Polycondensation of *N*-acetyl-*D*-glucosamine and structure of poly (*N*-acetyl-*D*-glucosamine)

Toshinobu Higashimura, Masahiko Sisido and Hiroshi Fuyama
Department of Polymer Chemistry, Kyoto University, Kyoto 606, Japan
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N-Acetyl-D-glucosamine was polymerized by the action of phosphorus pentoxide in dimethyl sulphoxide or poly(phosphoric acid ester) in dimethylformamide. The structure of the synthetic poly (*N*-acetyl-D-glucosamine) was determined by chemical methods. Polymers obtained from the phosphorus pentoxide/dimethyl sulphoxide system were found to contain predominantly α-1,4-glycosidic linkages, whereas polymers from the poly(phosphoric acid ester)/dimethylformamide system contained many β-1,6-linkages. These polymers showed different c.d. spectra and o.r.d. curves in the Cotton band of the amide chromophore, which was attributed to the difference in the position of glycosidic linkages. A definite correlation was found between the shape of the c.d. spectrum and the fraction of 1,4-glycosidic linkages estimated by the Morgan–Elson method. The observed trend was in agreement with the data for naturally existing glycosaminoglycanes. The spectroscopic data supported the conclusion of the structural analysis by chemical methods.

INTRODUCTION

The chemical synthesis of polysaccharides has been attempted by a variety of methods in the past few decades[1,2]. Among these attempts, acid catalysed polycondensation of monosaccharides in a strongly aprotic solvent is noteworthy. In this case monosaccharides are used without any chemical modifications and the reaction proceeds under relatively mild conditions. Because of these merits and some biological interest in the use of phosphoric acid derivatives, polycondensation has been extensively studied in dimethyl sulphoxide and dimethylformamide with phosphorus pentoxide and poly(phosphoric acid ester) as catalysts.

Schramm *et al.*[3] investigated the polycondensation of D-glucose in formamide with the use of poly(phosphoric acid ester) as catalyst and obtained a polymer of high molecular weight with a relatively high yield. They stated that the synthetic polyglucose contained β-1,4-glycosidic linkages exclusively. However, the high stereoselectivity seems doubtful in view of the nearly equal reactivity of C-2, C-3, and C-4 hydroxyl groups and the higher reactivity of C-6 group than the others[1]. Polycondensation has also been conducted effectively in dimethyl sulphoxide solution with phosphorus pentoxide as a dehydrating reagent. Micheel *et al.*[4] made extensive studies and obtained various polysaccharides with high molecular weight and a good yield. The polymers were found highly branched and the glycosyl residues appeared to be substituted randomly on C-2, C-3, C-4, and C-6 hydroxyl groups. Similar studies have been made by Husemann *et al.*[5], Mizuno[6], and Hirano *et al.*[7].

In the present study, polycondensation of *N*-acetyl-D-glucosamine was carried out by the use of phosphorus pentoxide and poly(phosphoric acid ester) as catalysts. Although polycondensation of *N*-acetyl-D-glucosamine has been reported by Micheel *et al.*[8] and Hirano *et al.*[7], the structure of the polymer was still unclear. Since the neighbouring group participation by a substituent on C-2 has been reported[9], a higher stereoselectivity may be expected in the polycondensation of *N*-acetyl-D-glucosamine than glucose. The structure of poly(*N*-acetylglucosamine) may be conveniently analysed by the use of c.d. spectra and o.r.d. curves around the Cotton band due to an amide chromophore. In this study these spectroscopic methods were first used to elucidate the position of glycosidic linkages of synthetic poly(*N*-acetylglucosamine).

EXPERIMENTAL

Materials

Commercial anhydrous *N*-acetyl-D-glucosamine (GNAc) (reagent grade) was used without further purification. Dimethyl sulphoxide (DMSO) and dimethylformamide (DMF) were fractionally distilled under reduced pressure immediately before use. Poly(phosphoric acid ethyl ester) (PPE) was obtained according to the method of Pollmann *et al.*[10]. Di-*N*-acetyl-chitobiose was obtained by the acetolysis of chitin followed by the deacylation of *O*-acetyl groups. The dimer was isolated by fractionation using a Biogel P-2 column.

Procedure for the polycondensation

Polycondensation with PPE and that with phosphorus pentoxide (P_2O_5) were carried out according to the method of Schramm *et al.*[3] and that of Mizuno[6], respectively. Polymerization was stopped by diluting the mixture with an equal amount of water and the solution was dialysed against running water for a week. After concentrating *in vacuo*, the solution was poured into ethanol, and the precipitated polymer was collected, washed with ethanol, and dried *in vacuo*.

Structural analysis of the polymer

The chemical structure of the polymer was determined by periodate oxidation[12] and the Morgan–Elson method[13].

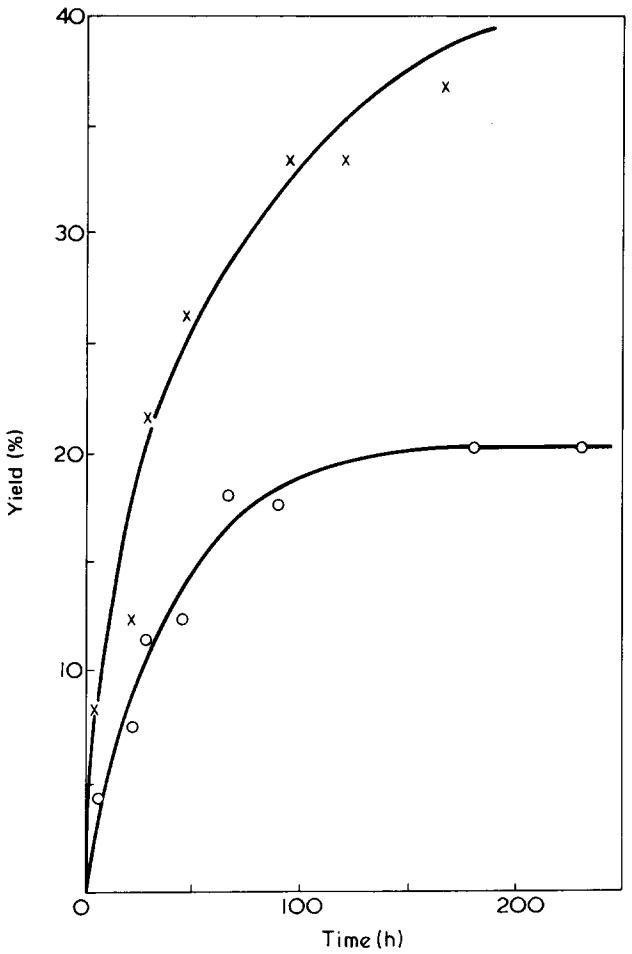

Figure 1 Time–yield curves for the polymerization of *N*-acetyl-D-glucosamine in P_2O_5/DMSO system at 20°C, [GNAc] = 0.1, [P_2O_5] = 0.1 (g/ml) (○) and in PPE/DMF system at 54°C, [GNAc] = 0.1, [PPE] = 0.2 (g/ml) (X)

In the latter case, the absorbance at 584 nm was used to estimate the fraction of 1,4-glycosidic linkages. Number-average molecular weight was measured with a Hitachi 117 vapour pressure osmometer and the values were compared with those calculated from the amount of terminal reducing residues measured by iodometry[14]. C.d. and o.r.d. were recorded in aqueous solution with a JASCO J-20 instrument. N.m.r. spectra were recorded on a JEOL MH-60 instrument.

RESULTS AND DISCUSSION

Polycondensation of N-*acetyl-*D-*glucosamine*

Polycondensations were carried out in P_2O_5/DMSO and PPE/DMF systems. *Figure 1* shows typical time–yield curves for the two systems. The reactions initially proceeded at similar rates and virtually stopped at about 20 or 40% yield. The final yield was larger in the PPE/DMF system. *Figure 2* shows the plot of the number-average degree of polymerization $\bar{n}$, measured by vapour pressure osmometry as a function of the reaction time. In the PPE/DMF system, $\bar{n}$ increased initially with time and finally took a value around 8. In P_2O_5/DMSO system, $\bar{n}$ was considerably lower than in the PPE/DMF system. In either system, the increase of polymer yield as well as the increase of degree of polymerization ceased after a few days. This implies that the polycondensation is accompanied by a reverse reaction and some termination reactions. In fact, a small amount of phosphorus (~4%) was detected in the polymer samples obtained by the two methods. Furthermore, Onodera *et al.*[15,16] reported the oxidation of glucose in the P_2O_5/DMSO system at higher temperatures around 60°C.

*Characterization of poly(*N-*acetyl-*D-*glucosamine) by chemical methods*

Polymer samples used for the structural study are listed in *Table 1*. The degree of polymerization was measured by vapour pressure osmometry and by the titration of the terminal reducing sugar residues. The data obtained by the two different methods agreed well, indicating the absence of any reactions which destroy the terminal reducing end. The position of glycosidic linkages was estimated by the Morgan–Elson method. The reaction product of 2-acetamide sugars in the Morgan–Elson test shows an absorption around 584 nm, provided that the hydroxyl group on C-4 position is *not* substituted. For example, ϵ_{584} for di-*N*-acetylchitobiose is only 7% of that for *N*-acetyl-D-glucosamine and the higher *N*-acetylated chitosaccharides give no detectable colour[17]. The synthetic polymers obtained from the P_2O_5/DMSO system showed a small absorption at 584 nm indicating the predominance of the 1,4-glycosidic linkage. On the other hand, a large absorption was found for the polymers from the PPE/DMF system. This result is consistent with the data of periodate oxidation. For linear poly(*N*-acetylglucosamine) with 1,4-glucosidic linkages, two moles of periodate ion are consumed for one mole of the polymer, and for the linear 1,6-polymer, $(n + 1)$ moles are consumed[18]. Therefore the data in *Table 1* indicate the predominance of the 1,4-glycosidic linkage for the polymers obtained from the P_2O_5/DMSO system and the 1,6-linkage for PPE/DMF system. The conclusion for the P_2O_5/DMSO system is in contrast with the result of polycondensations of D-glucose in the same system. In the latter case, the hydroxyl group on C-6 position was more

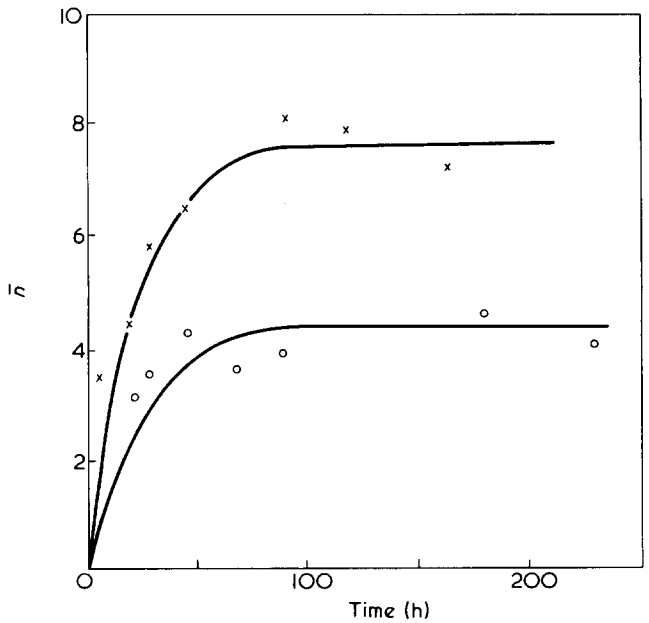

Figure 2 The plot of the number average degree of polymerization $\bar{n}$ as a function of the reaction time for the polymerization of *N*-acetyl-D-glucosamine in P_2O_5/DMSO system (○) and in PPE/DMF system (X). The polymer samples were the same as those used in *Figure 1*

Table 1 Characterization of poly(*N*-acetyl-D-glucosamines)

Sample number	Catalyst/ solvent	Temperature (°C)	$\bar{n}^a$	$\bar{n}^b$	$\epsilon_{584}{}^c$	$HIO_4{}^d$/ polymer	$[\alpha]_D{}^e$
GNAc[f]	–	–	1	–	4600	3.0	44
$(GNAc)_2$[g]	–	–	2.0	–	310	2.0	0
A-1	P_2O_5/DMSO	20	4.6	4.0	350	2.3	31
A-2	P_2O_5/DMSO	19	2.8	3.2	160	2.0	26
A-3	P_2O_5/DMSO	31	4.9	4.5	260	3.4	31
B-1	PPE/DMF	56	5.3	4.8	1200	4.8	9
B-2	PPE/DMF	58	5.5	–	1200	5.0	–

[a]Number-average degree of polymerization measured with vapour pressure osmometry; [b]number-average degree of polymerization measured with the titration of terminal reducing sugar residue; [c]the extinction coefficient at 584 nm in Morgan–Elson test; [d]molar consumption in HIO_4 oxidation; [e]specific rotation at 589 nm, deg/(g/ml)dm; [f]*N*-acetyl-D-glucosamine; [g]di-*N*-acetylchitobiose

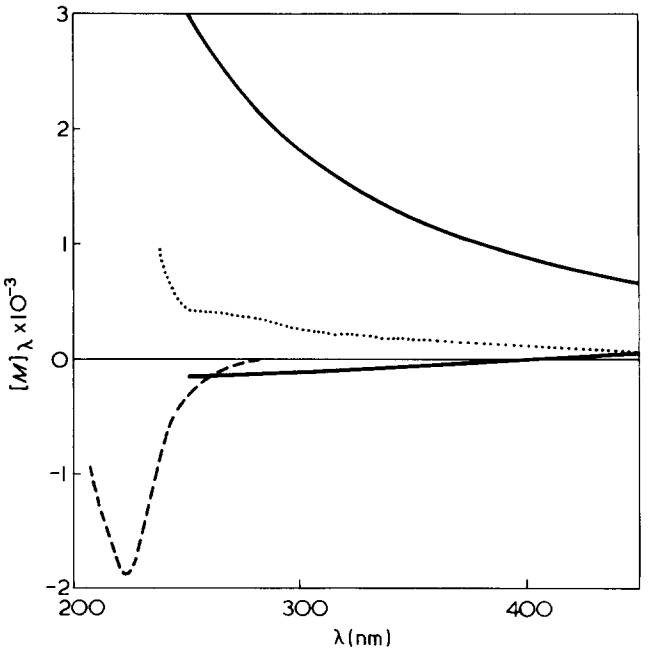

Figure 3 O.r.d. curves of amylose (——, upper curve), di-*N*-acetylchitobiose (– – –), synthetic poly(*N*-acetylglucosamine) obtained in P_2O_5/DMSO system at 20°C (A-1) (· · ·), and in the PPE/DMF system at 54°C (B-1) (——, lower curve)

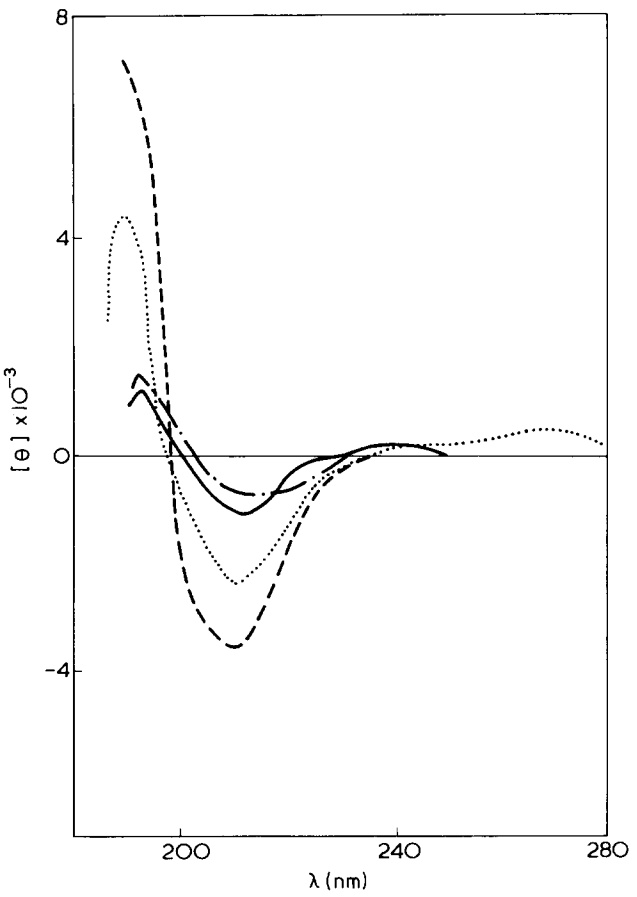

Figure 4 C.d. spectra of di-*N*-acetylchitobiose (– – –) and synthetic poly(*N*-acetylglucosamine) obtained in P_2O_5/DMSO system at 20°C (A-1) (· · ·), in the PPE/DMF system at 54°C (B-1) (——), and in the PPE/DMSO system at 55°C (–·–)

apt to undergo polycondensation than that on C-4[19]. The difference may be interpreted by the neighbouring participation of acetamide group on C-2[8].

Characterization by spectroscopic methods

Figures 3 and *4* show o.r.d. curves and c.d. spectra for various poly(*N*-acetylglucosamines), respectively. C.d. spectra show a negative Cotton band around 210 nm, while o.r.d. curves at longer wavelengths show positive dispersion, which is similar to the dispersion of amylose which contains no amide group. This indicates that the positive rotation at longer wavelengths is mainly due to the Cotton band of hemi-acetal or acetal group, which may be centred below 190 nm, and the effect of rotational strength of amide chromophore is small at longer wavelengths. Therefore, a large positive $[\alpha]_D$ indicates the presence of α-configuration and a small rotation indicates that of β-configuration. For glycosaminoglycanes with α-configuration, $[\alpha]_D$ is about $60 \sim 70°$[20]. The specific rotation for β-configuration may be estimated from chitin oligosaccharides to be $0 \sim 7°$[21]. The specific rotation in *Table 1* shows that α-configuration is predominant in the P_2O_5/DMSO system and β-configuration in the PPE/DMF system.

The amide Cotton band around 200 nm may be sensitive to the position of the glycosidic linkage. Stone[22] measured c.d. spectra of various naturally existing glycosaminoglycanes and suggested that the intensity of the positive c.d. band around 190 nm, θ_+, or its ratio to the intensity of the negative band around 210 nm, $|\theta_+/\theta_-|$, depended on the position of the glycosidic linkage irrespective of the configuration of the anomeric carbon (α or β). Polymers obtained from the P_2O_5/DMSO system showed a larger positive band at 190 nm than those from the PPE/DMF system. According to Stone[22], this indicates the predominance of 1,4-glycosidic linkage in the former samples and the result is in accordance with the results of the characterization by chemical methods. *Figure 5* shows the plot of the intensity

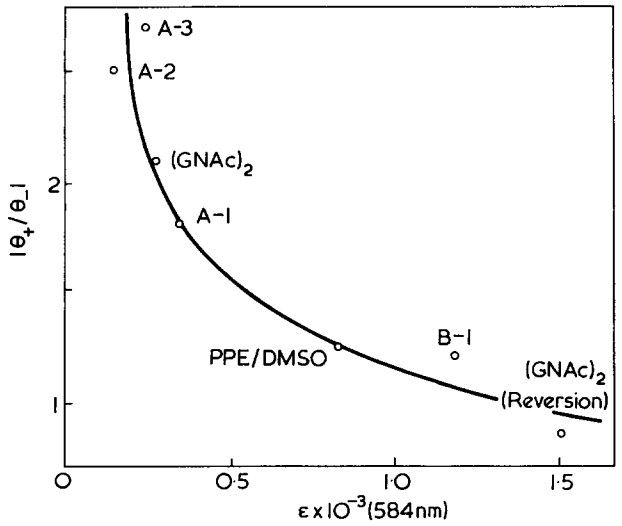

Figure 5 Plot of the intensity ratio of c.d. spectra for several oligo- and poly(*N*-acetylglucosamines) as a function of the extinction coefficient at 584 nm in the Morgan–Elson test. The sample numbers are indicated in the Figure. '$(GNAc)_2$(Reversion)' is an *N*-acetylglucosamine dimer obtained in the acid reversion[23]

ratio $|\theta_+/\theta_-|$ for several oligo- and poly(*N*-acetylglucosamines) against the extinction coefficient at 584 nm of the reaction product in the Morgan–Elson test. *Figure 5* includes the plots for a polymer obtained from the PPE/DMSO system and also an *N*-acetylglucosamine dimer obtained in the acid reversion[23]. A definite correlation was observed between the two quantities. Samples with smaller extinction coefficients, or with larger fractions of 1,4-glycosidic linkages, showed the larger intensity ratio. This result for the synthetic poly(*N*-acetylglucosamines) is in agreement with the result for naturally existing glycosaminoglycanes reported by Stone[22]. The above relation may be used for a rough estimation of the fraction of 1,4-glycosidic linkages from the shape of the c.d. spectrum.

The conformation of the pryanosyl ring (1C or C1) of the hexsosamine unit has been studied by n.m.r. spectroscopy[24]. The methyl signal of *N*-acetyl group in the equatorial position appears at δ = 1.9–2.05 ppm, whereas the signal for the axial position lies at 2.1 ppm in D_2O solution. All polymer samples obtained in the present study showed a single peak at 2.0 ppm, indicating that the *N*-acetylamido group is in the equatorial position and the conformation of the pyranosyl ring is C1.

I.r. spectra of the synthetic polymers were virtually identical with the spectrum of chitin. The relative intensity of carbonyl stretching peak of the amide group ($1650\ cm^{-1}$) was the same as that of chitin. This indicates that the amide groups remained unaffected during the polycondensation.

To conclude, *N*-acetylglucosamine was polymerized by two methods (P_2O_5/DMSO and PPE/DMF) and the structure of the polymer was determined by chemical and spectroscopic methods. Polymers obtained from the P_2O_5/DMSO system were found to contain principally α-1,4-glycosidic linkages, whereas polymers from the PPE/DMF system contained many β-1,6-linkages.

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